REMARKS

Claims 60, 61, 63, 64, 78-86 and 105-171 are currently pending; claims 78-86, 112 and 113 are allowed; claims 60, 63, 105, 109, 114-119 and 147 are rejected and claims 61, 64, 106-108, 110, 120-146 and 148-171 are objected to because they depend from rejected claims. Claims 60, 84, 105, 109, 114, 115, 119 and 147 are amended herein. Claim 60 and 84 are amended for clarity.

The Examiner has rejected claims 119 and 147 under 35 U.S.C §112, first paragraph for reciting new matter. Specifically, the examiner asserts that the as-filed specification does not support the phrase "wherein one or both primers of the second primer set do not anneal to an amplicon product produced by the first primer set in the PCR amplification," which was added to the claims in the previous amendment in order to distinguish over nested PCR reactions. In response, Applicants hereby amend claims 119 and 147 to recite language that is more clearly supported by the as-filed application. As is readily apparent from the as-filed specification, the multiplexed PCR reactions (production of two or more PCR amplicons in the same reaction vessel - see paragraph **[0051]** of the as-filed application) were intended to mean amplification of non-overlapping target sequences in a sample. As such, the application implicitly includes support for the currently-rejected language. Nevertheless, the claims are hereby amended to claim what is expressly described in this application - multiplexed PCR reactions performed on a sample of DNA (genomic, cDNA, etc.) comprising a first nucleic acid species containing a first target amplification sequence and a second nucleic acid species different from the first nucleic acid species containing a second target amplification sequence. Support, both express and implicit, for these amendments is found throughout the as-filed application, including, for example, in paragraphs **[0036]** (target nucleic acid species), **[0042]**, **[0048]**, **[0052]-[0054]**, **[0055]**, **[0060]**, **[0093]**, **[0141]**, and **[0146]**. In the context of the present disclosure, "species" refers to different nucleic acid classes in a reaction mixture, such as cDNAs generated from two different mRNAs and DNA fragments from two different sources, such as from two different alleles or chromosomal locations. As an example, cDNA prepared from two or more mRNA transcripts provides a DNA sample containing two or more different nucleic acid species with separate target amplification sequences. Arguably, after the first stage of a nested PCR reaction, the reaction sample contains two different DNA species (the original template and the first amplicon), but that reaction mixture is not subsequently subjected to a two-stage

amplification that is intended to modulate the relative accumulation of the two product amplicons of the first stage of the nested reaction and the nested reaction, as is required by all the pertinent claims.

The claims as they now stand require that two nucleic acids are present in the initial DNA sample, each having its own target amplification sequence. Nested PCR reactions, even though conducted in two parts, do not amplify sequences of different nucleic acid species both present in the DNA sample.

The Examiner has rejected claims 60, 63, 105 and 115-118 under 35 U.S.C. § 102(b) for anticipation by Takano *et al.* for two reasons. First, the Examiner has rejected claims 60, 63, 112 and 117 for reciting reverse transcription (RT) times of "less than about 10 minutes" and "about 2 minutes." The Examiner also has rejected claims 105, 115 and 118 for anticipation by Takano *et al.*, for purportedly reciting a multiplexed, multi-stage reaction.

The following is an excerpt from the portion of Takano *et al.* cited by the Examiner:

> "Reverse transcription was performed using either the whole RNA extracted by ABRP or 1μg of total RNA from tissue samples in an RT mixture containing ... in a total volume of 20μl at 37°C for ***60 min***. One microliter of first-strand cDNA was used as a template for the PCR reaction with specific primers for ***either*** RET (28), calcitonin (29), CEA (30), thyroglobulin (31), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA (32). The sequence of the primers were as follows: ... , Each reaction mixture consisted of 1μl cDNA, 0.5 μM of each primer, ... The reaction mixture was subjected to the PCR reaction. The conditions were as follows: for RET, calcitonin and CEA, 30 cycles of denaturation (94°C, 30 sec), annealing (60°C, 30 sec), and extension (72°C, 30 sec); for thyroglobulin and GAPDH, 35 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min), and extension (72°C, 1 min). After PCR amplification, 5μl of the reaction mixture was run on a 1.5% SeaKem GTG agarose gel (Takara) ..." (***emphasis added***).

Two things are to be noted in reference to this excerpt. First, the RT reaction time is 60 minutes, not ten or two minutes (or even "less than about 10" or "about 2" minutes, in case the Examiner is assuming, in making this rejection, that 60 minutes is even close or equal to "less

than about 10" or "about 2" minutes - an assumption that Applicants strenuously assert is entirely unreasonable). For this reason, the Examiner's basis for rejecting claims 60, 63, 112 and 117 is improper.

Second, the reactions were not multiplexed - two or more PCR reactions were not carried out in the same vessel (once again, see paragraph **[0051]** of the as-filed application). Applicants note that the excerpt above expressly states: "One microliter of first-strand cDNA was used as a template for the PCR reaction with specific primers for ***either*** RET (28), calcitonin (29), CEA (30), thyroglobulin (31), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA (32)." Use of the modifier "either" means that only one set of primers was used in each PCR reaction. Further, in reference to Figures 2 and 3, the gel photographs show only one amplicon each. If the reactions contained more than one amplicon, as would be the case in a multiplex reaction, the other amplicons present in the PCR reaction mixture would be seen in each gel. For example, reference is made particularly to Figures 2 and 3 and the "RET" and "Calcitonin" gels in which amplicons generated from both cDNAs should show up in the range of 267bp to 489bp in both the "RET" or "Calcitonin" photographs if the reactions were multiplexed because the multiplexed reaction would include both RET and calcitonin amplicons. Indeed, if the reactions were multiplexed, the DNA banding patterns in the "RET" and "Calcitonin" photographs would be identical within the depicted size range. They are not. This means that Takano *et al.* did not run one multiplexed reaction on a single gel and then either cut up the gel or cut up a photograph of the gel to generate the individual panels of Figures 2 and 3. As such, there is absolutely no hint of evidence that the reactions were multiplexed. The reactions were conducted separately as single-plex reactions. For these reasons, Applicants respectfully request withdrawal of the rejection of claims 63, 105 and 115-118 under 35 U.S.C. § 102(b) for anticipation by Takano *et al.*

The Examiner has rejected claim 105 under 35 U.S.C. § 102(b) for anticipation by Gerhard *et al.*, which recites a nested PCR reaction. Claim 105, as amended herein, does not recite a nested PCR reaction. At the outset, typical nested PCR reactions are not multiplexed. As stated in paragraph **[0051]** of the present as-filed application, a multiplexed method in its broadest sense "involves production of two or more amplicons in the same reaction vessel." In Gerhard *et al.*, two or more PCR reactions were not carried out in the same vessel. The process involved transfer of five microliters of the first reaction into a second tube for the second

reaction (page 726, column two, beginning 18 lines from bottom of the column). As stated above, claim 105 also requires that two different nucleic acid species are present in the initial DNA sample (for example and without limitation, different cDNAs or different genomic DNA fragments or alleles), each having its own target amplification sequence. Nested PCR reactions, even though conducted in two parts, do not amplify sequences of different nucleic acid species both present in the original DNA sample.

The Examiner has rejected claims 109 and 114 under 35 U.S.C. § 103(a) for obviousness over previously cited US Patent No. 5,985,552 (Howell *et al.*) in view of United States Patent No. 6,033,854 (Kurnit *et al.*). Specifically, the Examiner asserts that the nested reactions described in Howell *et al.* in light of the fluorescent detection methods of Kurnit *et al.*, render claims 109 and 114 obvious (though no mention is made of a fluorescent reporter in claim 114). As described above, Howell *et al.* does not describe a multiplexed reaction (as stated above, a multiplexed method in its broadest sense "involves production of two or more amplicons in the same reaction vessel). See, column 24, lines 5-12 of Howell *et al.* And there is no indication that the nested reactions described in column 24, lines 13-15 were conducted any differently - and no reason for the Examiner to presume that suddenly the reactions were performed sequentially in the same vessel without an express statement by Howell *et al.* that the reactions were multiplexed, as defined in the present application and as is commonly understood in the pertinent technical field. Further, claims 109 and 114 are hereby amended to remove the possibility that they read on two-step nested PCR configurations as described in the art cited by the Examiner.

In sum, Applicants hereby amend claims 105, 109, 114, 115, 119 and 147 to exclude nested PCR methods, whether or not conducted in multiple tubes, or in a single tube, as described in the newly-identified Ylitalo *et al.*, reference, described below and provided in connection with the following Information Disclosure Statement.

Information Disclosure Statement

Applicants, in accordance with the duty of disclosure pursuant to 37 C.F.R. § 1.56, hereby advise the United States Patent and Trademark Office of the references listed on the accompanying forms PTO/SB/08A and PTO/SB/08B "Information Disclosure Statement By Applicant," which were cited in an October 5, 2005 EPO search report in a related application.

The $180 fee for submission of this Information Disclosure Statement (IDS) under 37 C.F.R. §§1.97(c) and 1.17(p) is submitted herewith.

The Ylitalo *et al.* reference is noted because it discloses a single-tube nested PCR reaction in which primer annealing temperature is changed to alter the PCR reaction kinetics. The assay is non-quantitative and is simply used to type HPV. As described above, the claims, especially as they now stand, exclude nested PCR reactions. Further the teachings of the Ylitalo *et al.* reference as to a non-quantitative nested configuration cannot be predictably extended to quantitative multiplexed reactions. The template in a nested reaction, especially in the second stage of the nested reaction, is exponentially many-fold more prevalent than the templates found, for example, in a multiplexed reaction using cDNA product prepared from mRNA, and there is no requirement that the reaction be balanced in the sense that a quantitative multiplexed reaction is balanced.

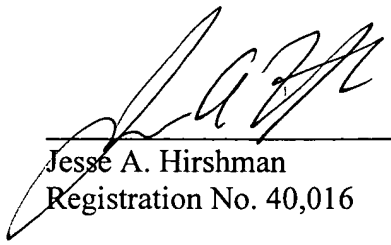
In a nested reaction there is no need to "balance" production of two or more different amplicons in order to accurately and precisely quantify production of the respective amplicons in a single reaction mix. Applicant's current claim language indicates that the multiplexed reaction is quantitative and that the reaction is "balanced," as is known in the art and as described, for example and without limitation, in paragraphs **[0008]**, **[0041]**, **[0052]** and **[0056]** of the as-filed application. In other words, the claims require that reactions proceed in a manner that permits relative amplification of the two or more amplification targets to yield Cts that accurately and precisely reflect the relative prevalence of the two amplification targets in the sample to be tested. This is not contemplated in Ylitalo *et al.* and the teachings of the present application do not follow from the teachings of Ylitalo *et al.*

Applicants note that the filing of this IDS shall not be construed to mean that the references presented herewith are prior art to the claims of the present application, and Applicant makes no such admission. Also, under 37 C.F.R. § 1.97(h), the filing of this IDS "shall not be construed to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in § 1.56(b)."

Applicant believes that claims 60, 61, 63, 64, 78-86 and 105-171 define over the prior art of record and are in proper form for allowance. Applicant respectfully requests allowance of claims 60, 61, 63, 64, 78-86 and 105-171. Applicant also requests that the Examiner call the

undersigned to discuss any additional questions or concerns with respect to the above-referenced patent application.

Respectfully submitted,



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